PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Docket No.: **Q92287**
Hanne MÜLLER et al	Conf. No.: **1130**
Appln. No.: **10/563,110**	Group Art Unit: **1627**
Filed: **June 19, 2006**	Examiner: **Betton, Timothy E**

For: **Lipids from methanotrophic bacteria for cholesterol reduction**

DECLARATION UNDER 37 C.F.R. § 1.132

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Anders Skrede, a Norwegian citizen, hereby declare as follows:

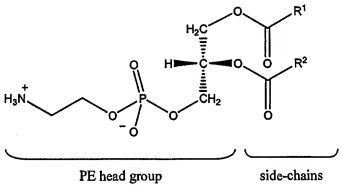
1. I am a co-inventor in respect of the patent application entitled "Lipids from methanotrophic bacteria for cholesterol reduction", filed on 2 July 2004 as PCT/GB2004/002866 and claiming priority from UK application No. 0315783.1 filed 4 July 2003. I understand this international application subsequently entered the United States national phase as application No. 10/563,110 (hereinafter "the Application"). I have read and am familiar with the content of the Application and I have also reviewed the claims which were filed on 22 October 2010 (hereinafter "the Claims").

2. I hold the position of Professor Emeritus at the Norwegian University of Life Sciences, having worked there since 1967 in the field of animal nutrition. Before retiring in 2008 I held the position of Head of Department in the Department of Animal and Aquacultural Sciences. Between 2002 and 2006 I was also a Section Leader and Deputy Director of the Aquaculture Protein Centre which consists of scientists from the Norwegian School of Veterinary Science, the Norwegian University of Life Sciences and Nofima AS. I have authored over 100 refereed

publications and have written a large number of book chapters, abstracts, proceedings at scientific international conferences and popular science articles. My research has focussed on the evaluation of feed ingredients for monogastric animals (including farmed fish, mink, fox, poultry and dogs), in particular evaluating feeds produced from methanotrophic bacteria grown on natural gas and which are employed in the present application. My work has also investigated the nutritional effects of different processing methods, such as fermentation, enzyme treatment, acid preservation, autoclaving, pelleting, expansion and extrusion of feeds. Much of my research has been financed by the Research Council of Norway in many cases in collaboration with industry.

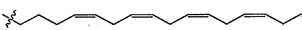
Technical background

3. Phosphatidylethanolamines (PEs), in common with phospholipids in general, are comprised of two parts; a PE "head" group attached to fatty acid side-chains. This may be illustrated as follows:



where R^1 and R^2 are fatty acid side-chain "tails".

4. Examples of fatty acid side-chains include the saturated palmitic acid ($C_{16:0}$) and the polyunsaturated EPA ($C_{20:5}$c,n-3), which correspond to the following R-groups:

palmitic acid: R = 

EPA: R =

5. Before the priority date of the Application, it was appreciated that the nature of the fatty acid side-chains will profoundly affect the cholesteremic response on administration of such a phospholipid to an animal. It was generally accepted in the art that administration of saturated and monounsaturated fatty acids (e.g. a PE containing $C_{16:0}$ side-chains) has a cholesterol increasing effect, whereas administration of polyunsaturated fatty acids (e.g. a PE containing EPA side-chains) has a cholesterol reducing effect.

6. PEs may comprise saturated, monounsaturated and/or polyunsaturated fatty acid side-chains. Not all PEs comprise EPA.

The Invention

7. The Application relates *inter alia* to a method of reducing cholesterol in an animal through oral administration of a composition comprising lipids obtained from methanotrophic bacteria. The composition recited in the Claims comprises methanotrophic bacterial phospholipids which have fatty acid side chains having between 14 and 22 carbon atoms. These fatty acid side chains are at least 80% by weight saturated or monounsaturated. In a preferred embodiment of the claimed invention, the phospholipids are phosphatidylethanolamines which have $C_{16:0}$ and/or $C_{16:1}$ side chains.

8. The lipid composition administered in the Example described in the Application is derived from a biomass (bacterial protein meal, or "BPM") which comprises a major portion of the methanotrophic bacterium *Methylococus capsulatus* (Bath). This lipid composition comprises predominantly phospholipids having $C_{16:0}$ and $C_{16:1}$ fatty acid side chains. There are no highly unsaturated fatty acids, e.g. Eicosapentaenoic acid, EPA (designated $C_{20:5}$c,n-3), or Docosahexaenoic acid, DHA, (designated $C_{22:6}$c,n-3) in the lipid composition used in the Example.

9. It is my view that the results described in the Examples of the Application indicate that administration of methanotrophic bacterial phospholipids comprising a major proportion of $C_{16:0}$ and $C_{16:1}$ fatty acid side chains can reduce the plasma cholesterol levels in animals, or maintain a reduced cholesterol level in animals, and

also that administration of the bacterial phospholipids can reduce the LDL:HDL cholesterol ratio in the plasma of animals.

10. Before the priority date of the Application, it was widely accepted that saturated fatty acids, especially $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$, have hypercholesterolemic properties, i.e. that administration of saturated fatty acids to animals causes an **increase** in plasma cholesterol levels. Relatively few studies on the monounsaturated fatty acids had been performed, but it was believed that certain monounsaturated fatty acids were hypercholesterolemic. In particular, the fatty acids $C_{14:1}$ (myristoleic acid) and $C_{16:1}$ (palmitoleic acid) were known to **elevate** plasma cholesterol levels when fed to pigs.

11. In contrast, before the priority date of the Application it was widely accepted that many polyunsaturated fatty acids (for example EPA and DHA) could reduce cholesterol levels in the serum of animals when administered orally.

12. Thus, at the priority date of the Application one would have expected a lipid composition comprising predominantly saturated and monounsaturated fatty acids (e.g. a phospholipid composition comprising a major proportion of $C_{16:0}$ and $C_{16:1}$ fatty acids) to have a hypercholesterolemic effect in an animal administered the composition, i.e. that the composition would be expected to **increase** plasma cholesterol levels on administration to an animal.

13. Before the priority date of the Application, studies had been carried out profiling the major fatty acid components in the membranes of methanotrophic bacteria (see e.g. Makula, *J. Bacteriol.* (1978) 134(3), pp.771-777; copy attached). In particular, the fatty acid composition of the phospholipid fraction of total lipids had been studied, because the phospholipid fraction makes up a major part of the total lipids in many species of methanotrophic bacteria.

14. It was known at that time that the phospholipid fraction of methanotrophic bacteria comprises a major portion of C_{14} to C_{22} fatty acid side-chains and that these side-chains are predominantly saturated or monounsaturated.

15. To my knowledge, no highly polyunsaturated fatty acids such as EPA or DHA have been identified in the phospholipid fraction of any methanotrophic bacteria. For example, Table 1 (see below) shows the total fatty acid composition of a biomass comprising a major portion of *Methylococus capsulatus* (Bath) and minor portions of *Ralstonia sp.* DB3, *Aneurinibacillus* sp. DB4 and *Brevibacillus agri* DB5. This biomass is the same as that used in the Example of the Application. The data in Table 1 show that the majority (over 85%) of fatty acids in the biomass are $C_{16:0}$ and $C_{16:1}$ fatty acids (7.5% of the fatty acids in the biomass were unidentified).

Nutrient	Mean	Range
Fatty acids [% of total fatty acids] (n = 4)*		
C12:0	0.1	0.0–0.1
C13:0	0.1	0.1–0.1
C14:0	4.2	3.9–4.4
C14:1n-5	0.5	0.1–1.0
C15:0	0.7	0.6–0.9
C16:0	49.2	48.1–51.1
C16:1	36.0	32.4–39.5
C17:0	0.5	0.2–1.1
C18:0	0.3	0.3–0.4
C18:1n-9	0.2	0.1–0.5
C18:1n-7	0.2	0.2–0.3
C18:2n-6	0.1	0.0–0.2
C18:3n-3	0.3	0.1–0.5
C20:1n-11,n-9	0.1	0.0–0.2

Table 1 - fatty acid composition of bacteria grown on natural gas

Summary

16. At the priority date of the Application, it was generally accepted in the art that administration of a lipid composition comprising C_{14} to C_{22} saturated fatty acids would have a hypercholesterolemic effect in animals. Some C_{14} to C_{22} monounsaturated fatty acids were also believed to have a hypercholesterolemic effect in animals. It was further known that administration of polyunsaturated fatty acids, e.g. EPA and DHA, could have a cholesterol-lowering effect on administration to animals.

17. Methanotrophic bacteria were known in the art to comprise a majority of C_{14} to C_{22} saturated and monounsaturated fatty acid side chains in the phospholipid fraction. Methanotrophic bacteria do not comprise EPA or DHA in the phospholipid fraction.

18. It would therefore have been expected at the filing date of the Application that oral administration of a composition comprising methanotrophic bacterial phospholipids as defined in the Claims would **increase** plasma cholesterol levels in animals. In light of this, it would have been unexpected that a composition as defined in the Claims is capable of **decreasing** serum plasma levels on oral administration to animals.

19. It is my opinion that the results of the experiments described in the Application go against the commonly-held belief in the art and could not have been confidently predicted at the priority date of the Application. It is therefore my opinion that the invention set out in the Claims would not have been obvious to a person of ordinary skill in the art at the priority date.

20. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Ås, Norway on the 7. day of January 2011

Anders SKREDE